Project 1010283

Biodegradation of PuEDTA and Impacts on Pu Mobility

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RESULTS TO DATE: This project, by Dr. Xun, supports work at PNNL (Bolton) regarding plutonium mobility in the subsurfac.. Ethylenediaminetetraacetate (EDTA) is a chelating agent that can increase the mobility of radionuclides and heavy metals in groundwater. Biodegradation of EDTA can decrease the enhanced mobility. The overall objective is to understand how microbial degradation affects Plutonium-EDTA transport in the environment and the specific objective of this component is to understand how microorganisms degrade EDTA. A chelating degrading bacterium BNC1 can use EDTA and nitrilotriacetate (NTA) as sole carbon and nitrogen sources. A gene cluster responsible for both EDTA and NTA degradation has been cloned and characterized (1,2). The same enzymes are used to degrade both compounds except that additional enzymes are required for EDTA degradation. Since the enzymes are located inside cells, EDTA and NTA must be transported into cells for degradation. For the first funding year, we have focused on how EDTA and NTA are transported into BNC1 cells. The EDTA-degrading gene cluster also contains genes encoding a hypothetical ABC-type transporter. We first demonstrated that the transporter genes and EDTA monooxygenase gene (emoA) were co-transcribed by RT-PCR, suggesting that the genes are involved in EDTA transport. We then characterized one of the gene product EppA. Using recombinant EppA purified from Escherichia coli, we have shown that EppA binds several metal:EDTA complexes by fluorescence techniques. In addition, EppA is shown to bind Mg:NTA, Ca:NTA and Fe(III):NTA but not free NTA. The fluorescence decrease at 365 nm was used to monitor Mg:EDTA binding in equilibrium titration experiments. This effort allows for the determination of the Kd (the equilibrium dissociation constant). Further experiments include determining the Kd for other EDTA and NTA metal complexes. The data suggest that BNC1 also uses the same ABC-type transporter for both EDTA and NTA uptake. A better understanding of the EDTA and NTA uptake in terms of the metal complexes and species will facilitate the development of bioremediation strategies in cleanup of radionuclide-EDTA contaminants.

DELIVERABLES: Publications: 1. Bohuslavek, J., J. Payne, Y. Liu, H. Bolton, Jr., and L. Xun. 2001. Cloning, sequencing and characterization of a gene cluster involved in EDTA degradation from the bacterium BNC1. Appl. Environ. Microbiol. 67:688-695. 2. Liu, Y., T. M. Louie, J. Payne, J. Bohuslavek, H. Bolton, Jr., and L. Xun. 2001. Identification, purification and characterization of iminodiacetate oxidase from the EDTA-degrading bacterium BNC1. Appl. Environ. Microbiol. 67:696-701.

COLLABORATIONS: This project has Dr. Luying Xun at Washington State University as a Co-PI investigating EDTA monooxygenase enzymology and genetics. We are collaborating with Dr. Bruce Rittmann (Northwestern University,) on developing a linked geochemical-microbial code to model chelating agent degradation.